Protein import into yeast microbodies.

Waterham, Hans Rudolf

SUMMARY OF THIS THESIS

PROTEIN IMPORT INTO YEAST MICROBODIES

A main characteristic of the eukaryotic cell is the compartmentalization of different metabolic processes into membrane-enclosed organelles. Each organelle contains a characteristic set of proteins to accomplish specific metabolic functions which are often essential for the cell's viability. The most recently discovered class of organelles includes the microbodies encompassing a group of organelles which have some morphological properties in common. Microbodies are ubiquitous in eukaryotic cells and can be subdivided in different types of organelles based on their metabolic functions (e.g. peroxisomes and glyoxysomes).

Yeasts are very well suited for studies on microbody biogenesis and function, since in these organisms the occurrence and specific metabolic function of the organelles can be precisely prescribed by manipulating the growth conditions. Furthermore, the development of molecular genetics for different yeast species have made the organisms readily accessible to multi-disciplinary studies on the molecular mechanisms underlying microbody biogenesis. The aim of the present work was to study these molecular mechanisms, with emphasis on the import of microbody proteins into their target organelles. The methylotrophic yeast *Hansenula polymorpha* was used as model organism; this organism represents one of the most extensively studied yeasts with respect to the biogenesis of microbodies. As a second model organism the methylotrophic yeast *Candida boidinii* was used. In contrast to *H. polymorpha* this yeast is able to grow on oleic acid, which induces the peroxisomal ß-oxidation pathway.

Chapter 1 summarizes the progress made in the last decade in the studies on different aspects of microbody protein import. Main breakthroughs were the identification of the two main peroxisomal protein targeting signals and the isolation of yeast mutants defective in the biogenesis of microbodies. Especially the availability of these mutants has opened the way to identify protein components involved in microbody protein import.

Chapter 2 presents immunocytochemical evidence for the *in vivo* acidic nature of the peroxisomal matrix in methylotrophic yeasts. For these studies the weak base DAMP was applied, which accumulates in acidic compartments and is recognized by specific antibodies. The accumulation of DAMP in peroxisomes was found to be related to the integrity of the cells. The implication of these observations are

discussed.

Chapter 3 describes the development and metabolic significance of peroxisomes in *C. boidinii*, when this yeast is cultivated on a mixture of two different carbon sources, each requiring an independent peroxisome-mediated pathway for its metabolism. Upon addition of methanol to oleic acid-grown cells in batch culture, only a part of the peroxisomal population present in one cell incorporated the newly synthesized matrix enzymes involved in methanol metabolism. Subsequent experiments with oleic acid-methanol-limited continuous cultures, however, showed that the enzymes of the ß-oxidation pathway and those involved in methanol-metabolism did coexist in one and the same organel, which gave rise to peroxisomes concurrently involved in the metabolism of the two different carbon sources. The results furthermore indicated that the heterogeneity observed in the peroxisomal population in the batch cultures is due to the fact that newly developed organelles are only temporarily competent to import newly synthesized proteins.

In chapter 4 the isolation and characterization of various mutants of *H. polymorpha* affected in peroxisome biogenesis are described. The mutants obtained were impaired to grow on methanol and had a characteristic feature in common: the cells contained several small peroxisomes although the bulk of the matrix protein resided in the cytosol. The obtained results suggest that these mutants are impaired in the protein import into peroxisomes (Pim$^-$ mutants). Genetic analysis revealed 5 different complementation groups which were designated *PER1-PER5*.

The development of efficient DNA transformation systems for *H. polymorpha* allowed to clone genes involved in microbody biogenesis by functional complementation of mutants which are affected in this biogenesis. In chapter 5 the cloning and sequencing of the *PER1* gene and the characterization of its gene product are described. The *PER1* gene codes for a 74 kDa peroxisomal matrix protein of relatively low abundance. The PER1 protein is the first protein described which contains both the established peroxisomal targeting signals PTS1 and PTS2; interestingly, both signals indeed do function in wild-type cells. The results obtained in this study indicate that import of the PER1 protein into *H. polymorpha* peroxisomes is a prerequisite to allow import of other matrix proteins. It is speculated that PER1 protein plays a role in controlling the protein import competence of the peroxisomes; the underlying mechanisms, however, remain to be solved.

Chapter 6 describes the isolation of temperature-sensitive peroxisome-deficient mutants of *H. polymorpha*. One of these mutants (*per13-6*^{ts}) was used in a study on the reintroduction of peroxisomes after a shift of cells from restrictive to permissive

temperatures. This mutant completely lacks peroxisomes (Per$^-$ phenotype) at restrictive temperature; at this temperature all the peroxisomal matrix proteins are localized in the cytosol. At permissive temperatures the cells show the wild-type phenotype and contain normal peroxisomes. After the temperature-shift new peroxisomes developed within one hour. No indication was found that the cytosolically localized peroxisomal matrix and membrane proteins, which were sythesized before the shift, were either incorporated or essential for the development of the new peroxisomes. These results indicated that alternative mechanisms of peroxisome biogenesis should be possible in addition to the generally accepted mode of multiplication by fission and growth from preexisting organelles.